academicJournals

Vol. 9(5), pp. 132-139, 3 February, 2015
DOI: 10.5897/JMPR2014.5141
Article Number: 8DCDE7550675
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro and *in vivo* evaluation of quinones from *Auxemma oncocalyx* Taub. on *Leishmania braziliensis*

Sayonara de Melo Viana[1], Maria Augusta D. Ferreira[2], Priscila Valera Guerra[1], Glauce S. Barros Viana[3] and Maria Jania Teixeira[1]*

[1]Departamento de Patologia e Medicina Legal, Faculdade de Medicina, Universidade Federal do Ceará (UFC), Rua Alexandre Baraúna, 949, 60.430-160 Fortaleza, CE, Brazil.
[2]Departamento de Farmácia, Faculdade de Farmácia, Odontologia e Enfermagem, UFC, Rua Capitão Francisco Pedro 1210, 60430-370 Fortaleza, CE, Brazil.
[3]Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, UFC, Av. Cel. Nunes de Melo, 1127, 60430-270 Fortaleza, CE, Brazil.

Received 25 June, 2013; Accepted 3 February, 2015

The effect of a quinone fraction (QF) isolated from the heartwood of *Auxemma oncocalyx* Taub. was investigated *in vitro* and *in vivo* on *Leishmania braziliensis*. Although QF (1-10 µg/mL) showing marked *in vitro* anti-*Leishmania* activity (81 to 94%), the oral treatment with the compound did not protect hamsters against progressive *L. braziliensis* infection. When QF was administered intraperitoneally (20 mg/kg) the lesion size was reduced by 65%; however, it was not able to promote parasite eradication, as evidenced by the high number of parasites in draining lymph nodes. Quinones are highly redox active molecules and with their semiquinones radicals can lead to formation of reactive oxygen species (ROS). The generation of ROS could probably explain the *in vitro* leishmanicidal activity of the quinones, since promastigotes are susceptible to H_2O_2 lethal effect *in vitro*. In conclusion, although quinones seem to be effective against *Leishmania* parasites *in vitro*, they do not demonstrate a therapeutic effect in experimental leishmaniasis. In addition, it can be hypothesized that QF *in vivo* might possibly be converted into non-active metabolite(s) or be inactivated either by reduction or by interaction with serum proteins, losing its leishmanicidal activity.

Key words: *Leishmania braziliensis*, *Auxemma oncocalyx*, quinone, hamster, cutaneous leishmaniasis.

INTRODUCTION

Leishmaniasis are protozoan diseases which represent a risk for 350 million people worldwide, and 2 million new cases occur yearly (World Health Organization [WHO], 2010). Etiologic agents are intracellular parasites of the genus *Leishmania* that display a spectrum of a manifestation which goes from cutaneous involvement ith late destruction of mucous membranes to generalized systemic visceral disease with fatal outcome, if not treated (Pearson et al., 2000; Desjeux, 2004). There is still no effective vaccine to control the wide range of disease caused by different species of *Leishmania* (Oliveira et al., 2009). The disease may regress spontaneously or evolve,

*Corresponding author. E-mail: mjteixeira601@gmail.com. Tel: +55-85-33668311. Fax: +55-85-33668316.

thus requiring treatment (Piscopo and Mallia, 2006). The antileishmanial first-line drugs are the pentavalent antimonials, meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®). They are generally effective during the acute infection stages but not against the late stages, and produce significant side effects due to high toxicity and tissue drug accumulation, which includes myalgias, nausea, vomiting, cardiac arrhythmia, hepatitis, or pancreatitis (Croft et al., 2006; Palumbo, 2009). Amphotericin B and pentamidine are better tolerated drugs, but require long courses of parenteral administration (Amato et al., 2008). Miltefosine and fluconazole have recently showed effectiveness against cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* (Machado et al., 2010; Sousa et al., 2011), but despite the lower toxicity, these second line drugs are not useful against other forms of leishmaniasis (Palumbo, 2009). Furthermore, the continuous use of ineffective drugs has led to the development of resistance to their compounds (Escobar et al., 2001), which have stirred an urgent need for novel, effective, and safe drugs for treatment of leishmaniasis.

Since plant derivatives are among the most active agents against infections (Delorenzi et al., 2001), many researchers have been looking for better effects and less toxicity to treat leishmaniasis in these substances, especially those already used by people living in endemic areas (Weniger et al., 2001). *Auxemma oncocalyx* Taub. belongs to the Boraginaceae family, it is known as "pau branco" (white wood), and is easily available by rural settlers in Northeastern Brazil. The stem bark of the tree is astringent and popularly used in the treatment of wounds (Braga, 1976).

Previous pharmacological studies reported that the hydroalcoholic extract of the stem presents antioxidant, analgesic and anti-inflammatory properties (Ferreira et al., 2004, 2008). Alantoin and β-sitosterol glycoside have been isolated from *A. oncocalyx* and are responsible for some of its pharmacological properties (Pessoa and De Lemos, 1997). Besides these compounds, at least six quinones were successfully isolated and oncocalyxone A, obtained in significant amounts, seems to be partially involved in the bioactivity of the plant (Leyva et al., 2000). Several quinones present antileishmanial (Sauvain et al., 1993; Sittie et al., 1999; Teixeira et al., 2001), antitumoral (Morello et al., 1995; Itoigawa et al., 2003), antifungal (Perry et al., 1991; Gafner et al., 1996), or antimalarial activity (Figueiredo et al., 1998), either *in vitro* or *in vivo.* The aim of the present work was to evaluate the *in vitro* and *in vivo* activity of the quinone fraction from *A. oncocalyx* Taub. against *L. braziliensis*.

MATERIALS AND METHODS

Plant extraction and purification of the quinone fraction

A. oncocalyx Taub. was collected in the city of Pentecoste, Ceará State, Northeastern Brazil, and identified by Prof. A. G. Fernandes from the Biological Sciences Department. A voucher specimen has been deposited under the number 18459 at the Prisco Bezerra Herbarium of the Universidade Federal do Ceará, Brazil. The quinone fraction (QF) was prepared from grinded heartwood ethanolic extract through exhaustive aqueous extraction followed by lyophilization. Hydrosoluble components contained around 80% of oncocalyxone A, according to a previous characterization (Pessoa et al., 1993).

Parasites and animals

Three to four months adult female and male golden hamsters (*Mesocricetus auratus*), weighing 80 to 90 g, obtained from the central animal facility of Departamento de Patologia e Medicina Legal of Universidade Federal do Ceará (DPML/UFC), and housed in groups of six to eight per cage with free access to water and food. The Animal Care and Utilization Committee from UFC approved all experimental procedures (process n° 65/08). The *L. braziliensis* (MHOM/BR/94/H-3227) was originally isolated from skin lesions of a patient with CL from Ceará State, and previously typed using isoenzymes electrophoresis and monoclonal antibodies (De Oliveira et al., 2004).

The parasites, stored in liquid nitrogen, were thawed and cultured as promastigotes at 26°C in Schneider's insect medium (Sigma-Aldrich, Chemical Co., St. Louis, USA) supplemented with 10% heat-inactivated fetal calf serum (Sigma), 2% sterile normal human urine, 2 mM L-glutamine (Gibco BRL, Grand Island, NY), and antibiotics [100 U/ml penicillin, 100 µg/ml streptomycin sulfate (Sigma-Aldrich)]. Subcultures were made in the stationary phase of growth and parasites were used at no later than the fourth passage. Prior to infection, promastigotes were harvested from culture, washed in sterile saline, counted in Neubauer's chamber and adjusted to the appropriate concentration.

Anti-promastigote activity

For the tests *in vitro* against promastigotes in 96-well plates stationary-phase promastigotes were added at a concentration of 10^6 cells/well in Schneider medium supplemented with 10% heat-inactivated fetal calf serum (Sigma), 2% sterile normal human urine, 2 mM L-glutamine (Gibco), and 100 U/ml penicillin + 100 µg/ml streptomycin sulfate (Sigma-Aldrich) determined after counting in a Neubauer chamber. Drugs were diluted with DMSO 5% and placed at concentrations of 0.01 to 10 µg/mL. Amphotericin B was chosen as the control drug. The plates were incubated in a biochemical oxygen demand (BOD) at 24°C, and after that, 1 µCi per well of [3H]thymidine (Amersham International, Amersham, UK) was added and the cells were incubated for another 24 h and harvested. [3H]thymidine incorporation was measured in a β-counter (Pharmacia, Finland) after washing to distinguish the non-used thymidine from the one incorporated in DNA. Assays were done in duplicate and made a replica of each test. The inhibition of growth was expressed as the percent decrease of radioactive incorporation in treated cells when compared with untreated control. It was considered a good antileishmanial activity when values equal or greater than 70% were obtained. The formula for calculation of antileishmanial activity was:

$$\frac{\text{cpm of control cells (promastigotes) - cpm of treated cells (promastigotes + drugs)}}{\text{cpm of control cells (promastigotes) x 100}}$$

Infection, treatment and lesion development

Hamsters were infected subcutaneously in the right hind footpad with 10^6 stationary phase *L. braziliensis* promastigotes in 20 µl of sterile saline. Treatment was initiated two weeks after inoculation when lesions were well defined. Animals were randomly divided into groups of six to eight and the drugs administered daily for 28 consecutive days. QF was dissolved in distilled water containing 0.1% of Tween 80 and 0.5% of carboxymethylcellulose (CMC) and administered using the following routes: (a) oral (p.o.), with 10 or 20 mg/kg by intragastric intubation; (b) intraperitoneal (i.p.) with 20 mg/kg. Alternatively, Glucantime® (Sanofi-Aventis Farmacêutica, São Paulo, Brazil) was injected at the dose of 60 mg/kg/day intramuscularly (i.m.). Control groups received p.o. or i.p. drugless vehicle (Tween 80 + CMC + H_2O) at equivalent volumes. Before treatment was conducted, a toxicity test using various concentrations of QF that demonstrated concentrations of 10 and 20 mg/kg did not kill the animals. Lesion sizes were measured weekly with a dial gauge caliper (Mitutoyo, 0.01 mm sensitivity) and expressed as the difference between the thicknesses (mm) of the infected and contralateral uninfected footpads.

Treatment outcome

The number of parasites in the popliteal lymph was quantified by the limiting dilution technique as previously described (Lima et al., 1997). Briefly, after the treatment, the animals were euthanized by inhalation of halothane (Sigma-Aldrich) and submerged in 3% iodized alcohol up to 3 min to allow decontamination. The lymph nodes were removed aseptically and macerated in a Petri dish with 2 ml of Schneider medium. After removal of debris by sedimentation for 5 min, the homogenates were serially diluted (1:10) in Schneider's medium supplemented with 100 U/ml of penicillin/ml, 100 µg/ml of streptomycin/ml, 10% fetal calf serum and 2% sterile human urine. One hundred microliters of these dilutions was distributed into 96-well flat bottom plates containing agar-blood in 6 replicates per concentration. The plates were incubated at 25°C and observed under an inverted microscope (Nikkon, Japan) every 3 days, up to a maximum of 30 days, to record the dilutions containing promastigotes. The final number of parasites per tissue was determined using the ELIDA software, version 12c for window (Taswell, 1984).

Statistical analysis

The data are presented as mean ± standard error of the mean. The significance of the results relating to the parasite load was calculated by Mann-Whitney test. The anti-promastigota activity and lesion sizes from treated and untreated animals were analyzed by the one-way analysis of variance (ANOVA) and complemented by the Bonferroni test for multiple comparisons. All analysis and graphs were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, USA). Values of $P < 0.05$ were considered significant.

RESULTS

QF displayed marked *in vitro* anti-promastigote activity at concentration of 1 and 10 µg/mL (80.5 ± 2.80 and 91.0 ± 0.65%, respectively) against *L. braziliensis*, being similar to Amphotericin B (Figure 1). To determine the effect of QF on *L. braziliensis* infection in hamsters, the animals days via oral. The results showed that hamsters treated with QF (10 or 20 mg/kg, p.o.) did not present significant reduction of the lesion thickness as compared to untreated animals. Also, doses of 20 and 10 mg/kg (p.o.), did not produce significant reduction of parasite load in lymph nodes as compared to untreated group (Figure 2A and B). As expected, animals treated with glucantime showed antimonial drug effectively brought footpad sizes down to normal within 2 weeks of treatment and also suppressed parasite growth in lymph nodes (Figure 2A and B).

To investigate if a different administration route of the drug could be more effective on controlling *L. braziliensis* infection, hamsters were treated with 20 mg/kg QF intraperitoneally for 28 days (the highest dose, but yet not toxic to animals). The results showed that the lesion size of hamsters treated with QF i.p. decreased by 65% ($P < 0.05$), and parasite burden in the lymph nodes was significantly lower ($9.3 \pm 3.5 \times 10^5$) when compared with untreated controls ($1.98 \pm 2.0 \times 10^6$) (Figure 3A and B).

DISCUSSION

Antileishmanial activity has been reported in several compounds extracted from medicinal plants belonging to diverse chemical groups, including quinones (Chan-Bacab et al., 2001; Rocha et al., 2005). Other studies have found simple quinones isolated from dried trunks of *Jacaranda copaia* to present significant anti-promastigote and anti-amastigote activities *in vitro* against *L. amazonensis*, but only weak activity when tested against *L. amazonensis*-induced lesion in mice (Sauvain et al., 1993). Benzoquinones were found to be active *in vitro* against trypanosomes, but this has not been confirmed *in vivo* (Grady et al., 1984; Pahn et al., 1988).

In murine model, *Leishmania major*- and *Leishmania donovani*-infected BALB/c treated with buparvaquone formulation (BPQ), showed parasite burden decrease in lesions and liver, smaller and not ulcerated lesions, in comparison with untreated control (Garnier et al., 2007). Recently, a study using the molecular hybridization of a naphthoquinone core with a pterocarpan moiety (LGB-118) led to significant reduction in skin lesions development, swelling, ulceration and parasite burden of BALB/c *L. amazonensis*-infected (Da Cunha-Júnior et al., 2011). Also, acetylisolapachol, a hydroxyquinone derivative, showed *in vitro* activity against *L. braziliensis,* and *in vitro* and *in vivo* against *L. amazonensis* (Lima et al., 2004).

The leishmanicidal activity of a drug may be selective and direct against the parasite, or it may act indirectly by activating macrophage microbicidal mechanisms for instance. According to *in vitro* model systems, the macrophage microbicidal response to *Leishmania* infection can follow two distinct pathways. Upon infection, promastigotes elicit a respiratory burst with the generation of reactive oxygen intermediates such as hydrogen peroxide (H_2O_2), $^{\bullet}OH$ radical, superoxide ($^{\bullet}O_2^-$) were inoculated with promastigotes and treatment for 28

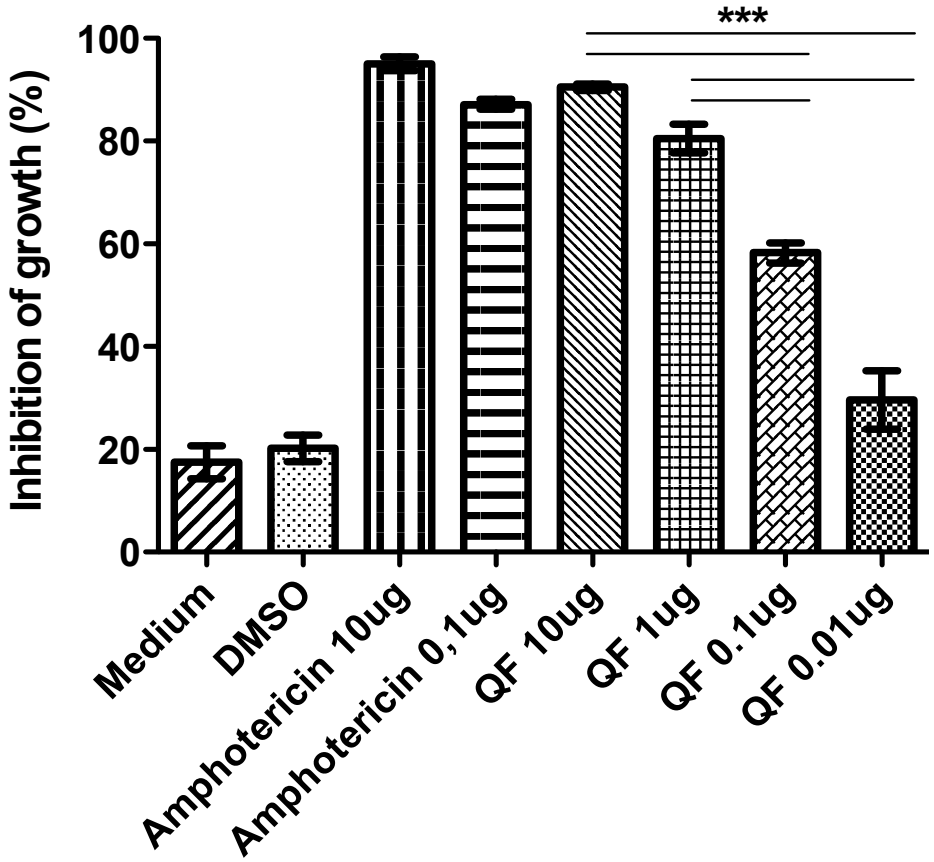

Figure 1. Antileishmanial activity *in vitro* of a quinone fraction (QF) isolated from the heartwood of *Auxemma oncocalyx* Taub. against promastigores of *L. braziliensis*. The inhibition of growth was expressed as the percent decrease of radioactive incorporation in treated parasites when compared with untreated control. ***P < 0.05 (test ANOVA).

and peroxynitrate as part of an oxygen dependent mechanism to kill promastigotes, however, a small percentage of phagocytosed organisms can survive (Beaman and Beaman, 1984). Second, murine or human macrophages can be activated to kill intracellular amastigotes, the form present during established infection, by previous exposure to cytokines such as IFN-γ and TNF-α, which activate both oxidative and non-H_2O_2-associated microbicidal mechanisms (Bogdan et al., 1990; Liew, 1992; Assreuy et al., 1994; McSorley et al., 1996; Panaro et al., 1999). Studies have demonstrated that both H_2O_2-associated and non-H_2O_2-associated pathways contribute to *Leishmania* killing and that their relative degree of importance may differ during initial promastigote invasion versus established amastigote infection (Chang, 1983; Murray and Nathan, 1999; Erel et al., 1999).

Quinones are highly redox active molecules and with their semiquinones radicals can lead to formation of reactive oxygen species (ROS), including $^{\bullet}O_2^-$, H_2O_2, and ultimately to hydroxyl radicals (Bolton et al*.,* 2000). The formation of ROS could probably explain the *in vitro* leishmanicidal activity of the quinones in this study, since promastigotes are readily susceptible to killing by H_2O_2 *in vitro* (Murray, 1981; Zarley et al., 1991). It has been shown that *Leishmania chagasi* promastigotes *in vitro* are susceptible to killing by both H_2O_2 and the redox-cycling compound menadione, a quinone that causes the generation of $^{\bullet}O_2^-$ in the presence of promastigotes (Wilson et al., 1994). However, this source of quinone free radicals seems to be often more readily apparent *in vitro* than *in vivo*. Menadione providing an excellent example of this phenomenon, although presents anticancer activity *in vitro* in combination with other chemotherapeutic agents. However, menadione does not demonstrate the same activity *in vivo* even at high doses (Nestor et al., 1991; Djuric et al., 1995). Besides, in a BALB/c mouse model of leishmaniasis, sublethal concentrations of menadione caused *L. chagasi* promastigotes to become more virulent (Wilson et al., 1994).

When QF was administered intraperitoneally at a dose of 20 mg/kg, the lesion size was reduced by 65%, but it was however not able to promote parasite killing as evidenced by the mean number of parasites in the lymph nodes as compared to controls. Reduction in the lesion size induced by *Leishmania* infection can not necessarily

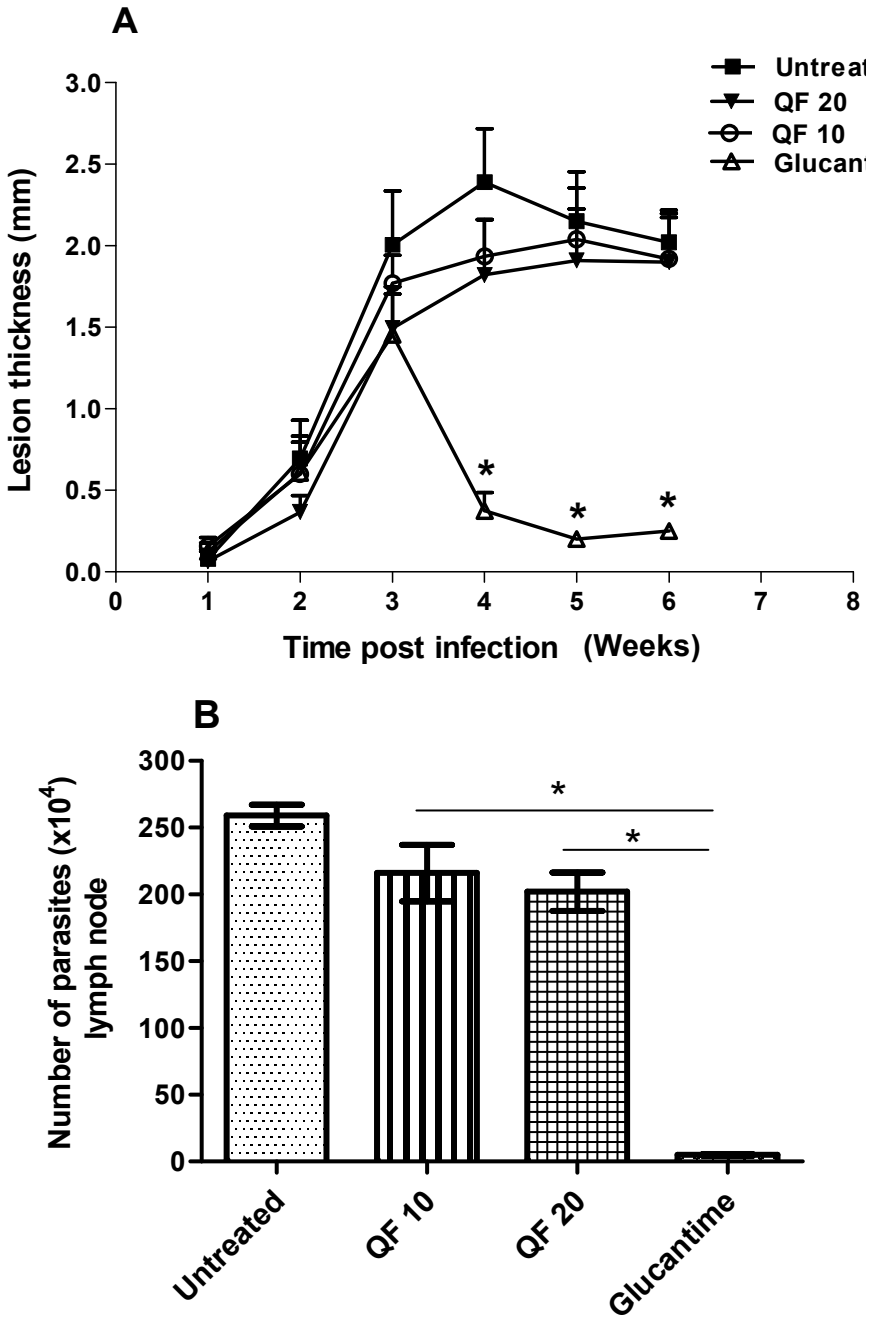

Figure 2. Effect of oral treatment with QF (20 or 10 mg/kg). (A) Lesion growth, and (B) Parasite load in lymph node. Hamsters were infected with 10^6 *L. braziliensis* promastigotes (8 per group). Animals were left untreated or were treated with daily dose of 20 mg/kg QF or with 10 mg/kg or 60 mg/kg/i.m. glucantime. Treatment started three weeks post infection. Lesion thickness was measured weekly using a dial gauge caliper (mean ± SE, n = 8). Parasite load was evaluated after the treatment. *P < 0.05 (A, Glucantime versus untreated or QF treatment; B, Glucantime versus QF treatment).

mean a decrease in the parasite load, but only a decrease of the local inflammatory reaction. QF of *A. oncocalyx* has a wide range of biological effects including anti-parasitic, antitumoral and antiplatelet activities (Leyva et al., 2000; Ferreira et al., 2008). It have also been shown that the compound presents anti-inflammatory and antiedematogenic activities, reducing the effect of carrageenan (Ferreira et al., 2004). Therefore, it

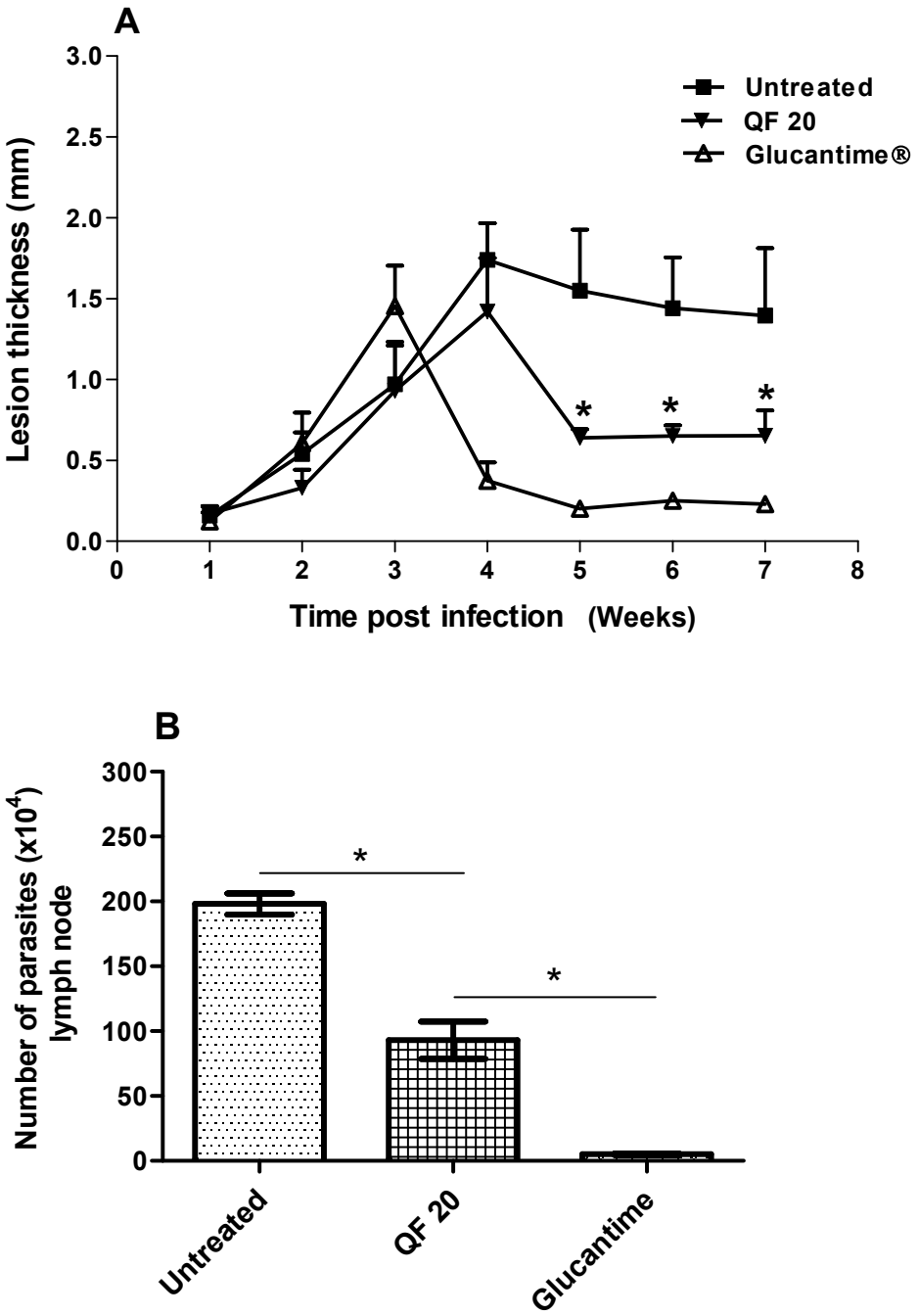

Figure 3. Effect of intraperitoneal treatment with QF (20 mg/kg). (A) Lesion growth, and (B) Parasite load in lymph node. Hamsters were infected with 10^6 *L. braziliensis* promastigotes (6 per group). Animals were left untreated or were treated with daily dose of 20 mg/kg QF or 60 mg/kg/i.m. glucantime. Treatment started three weeks post infection. Lesion thickness was measured weekly using a dial gauge caliper (mean ± SE, n = 8). Parasite load was evaluated after the treatment. *P < 0.05 (A, QF treatment versus untreated; B, QF treatment versus untreated or Glucantime).

is suggested that the reduction in the lesion size produced by 20mg/kg, i.p of QF in *L. braziliensis*-infected hamsters may probably be due to its anti-inflammatory effect. In addition, the observation that QF when used via oral did not offer significant protection in hamsters infected by *L. braziliensis*, suggests that QF can possibly be converted into non-active metabolite(s) or otherwise be inactivated either by reduction or by interaction with

serum proteins. Quinones can be metabolized by various routes: substitution or reductive addition with nucleophilic compounds or one and two-electron reductions (Koster, 1991). Driscoll et al. (1974) found that the biological activity of some quinones, as lapachol and its analogs, is directly related to their chemical structures, thus any structural alteration *in vivo* will result in an inactive product or will abolish their biological activities (Teixeira et al., 2001).

Despite QF not to have demonstrated an anti-parasitic effect in *L. braziliensis*-infected hamsters, the production of quinones derivatives might insure their interest as antileishmanial candidate drugs. Furthermore, alternative therapy derived from medicinal plants opens new perspectives towards the development of effective, readily available and less-costly drugs for the treatment of the leishmaniasis in endemic areas.

Conflict of interest

The authors report no declarations of interest.

ACKNOWLEDGEMENT

The authors thank Otília D. L. Pessoa and Telma L. G. Lemos from Departamento de Química Orgânica e Inorgânica, UFC, Fortaleza, CE, Brazil. This work was partially supported by the Brazilian National Research Council (CNPq).

REFERENCES

Amato VS, Tuon FF, Bacha HA, Neto VA, Nicodemo AC (2008). Mucosal leishmaniasis. Current scenario and prospects for treatment. Acta Trop. 105(1):1-9.

Assreuy J, Cunha FQ, Epperlein M, Noronha-Dutra A, O'Donnell CA, Liew FY, Moncada S (1994). Production of nitric oxide and superoxide by activated macrophages and killing of *Leishmania major*. Eur. J. Immunol. 24(3):672-676.

Beaman L, Beaman BL (1984). The role of oxygen and its derivatives in microbial pathogenesis and host defense. Annu. Rev. Microbiol. 38:27-48.

Bogdan C, Moll H, Solbach W, Röllinghoff M (1990). Tumor necrosis factor-α in combination with interferon-γ, but not with interleukin 4 activates macrophages for elimination of *Leishmania major* amastigotes. Eur. J. Immunol. 20(5):1131-5.

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000). Role of quinones in toxicology. Chem. Res. Toxicol. 13(3):135-60.

Braga R (1976). Plantas do Nordeste, especialmente do Ceará. 3. ed. Fortaleza: ESAM, p. 510.

Chan-Bacab MJ, Peña-Rodríguez LM (2001). Plant natural products with leishmanicidal activity. Nat. Prod. Rep. 18(6):674-688.

Chang KP (1983). Cellular and molecular mechanisms of intracellular symbiosis in leishmaniasis. Int. Rev. Cytol. Suppl. 14:267-305.

Croft SL, Seifert K, Yardley V (2006). Current scenario of drug development for leishmaniasis. Indian J. Med. Res. 123(3):399-410.

Da Cunha-Júnior EF, Pacienza-Lima W, Ribeiro GA, Netto CD, do Canto-Cavalheiro MM, da Silva AJ, Costa PR, Rossi-Bergmann B, Torres-Santos EC (2011). Effectiveness of the local or oral delivery of the novel naphthopterocarpanquinone LQB-118 against cutaneous leishmaniasis. J. Antimicrob. Chemother. 66(7):1555-1559.

De Oliveira CI, Teixeira MJ, Teixeira CR, Ramos de Jesus J, Bomura Rosato A, Santa da Silva J, Brodskyn C, Barral-Netto M, Barral A (2004). *Leishmania braziliensis* isolates differing at the genome level display distinctive features in BALB/c mice. Microbes Infect. 6(11):977-984.

Delorenzi JC, Attias M, Gattass CR, Andrade M, Rezende C, da Cunha Pinto A, Henriques AT, Bou-Habib DC, Saraiva EM (2001). Antileishmanial activity of an indole alkaloid from *Peschiera australis*. Antimicrob. Agents Chemother. 45(5):1349-1354.

Desjeux P (2004). Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. 27(5):305-318.

Djuric Z, Corbett TH, Valeriote FA, Heilbrun LK, Baker LH (1995). Detoxification ability and toxicity of quinones in mouse and human tumor cell lines used for anticancer drug screening. Cancer Chemother. Pharmacol. 36(1):20-26.

Driscoll JS, Hazard GF Jr, Wood HB Jr, Goldin A (1974). Structure-antitumor activity relationships among quinone derivatives. Cancer Chemother. Rep. 4(2):1-362.

Erel O, Kocyigit A, Bulut V, Gurel MS (1999). Reactive nitrogen and oxygen intermediates in patients with cutaneous leishmaniasis. Mem. Inst. Oswaldo Cruz. 94(2):179-183.

Escobar P, Yardley V, Croft SL (2001). Activities of hexadecylphosphocholine (miltefosine), AmBisome, and sodium stibogluconate (Pentostam) against *Leishmania donovani* in immunodeficient *scid* mice. Antimicrob. Agents Chemother. 45(6):1872-1875.

Ferreira MA, do Nascimento NR, de Sousa CM, Pessoa OD, de Lemos TL, Ventura JS, Schattner M, Chudzinski-Tavassi AM (2008). Oncocalyxone A inhibits human platelet aggregation by increasing cGMP and by binding to GP Ibα glycoprotein. Br. J. Pharmacol. 154(6):1216-1224.

Ferreira MA, Nunes OD, Fontenele JB, Pessoa OD, Lemos TL, Viana GS (2004). Analgesic and anti-inflammatory activities of a fraction rich in oncocalyxone A isolated from Auxemma oncocalyx. Phytomedicine 11(4):315-322.

Figueiredo JN, Räz B, Séquin U (1998). Novel quinone methides from *Salacia kraussii* with in vitro antimalarial activity. J. Nat. Prod. 61(6):718-723.

Gafner S, Wolfender JL, Nianga M, Stoeckli-Evans H, Hostettmann K (1996). Antifungal and antibacterial naphthoquinones from *Newboudia laevis* roots. Phytochemistry 42(5):1315-1320.

Garnier T, Mäntylä A, Järvinen T, Lawrence J, Brown M, Croft S (2007). *In vivo* studies on the antileishmanial activity of buparvaquone and its prodrugs. J. Antimicrob. Chemother. 60(4):802-810.

Grady RW, Blobstein SH, Meshnick SR, Ulrich PC, Cerami A, Amirmoazzami J, Hodnett EM (1984). The *in vitro* trypanocidal activity of N-substituted p-benzoquinones imines: assessment of biochemical structure-activity relationships using the Hansch approach. J. Cell Biochem. 25(1):15-29.

Itoigawa M, Kashiwada Y, Ito C, Furukawa H, Tachibana Y, Bastow KF, Lee KH (2003). Antitumor agents. 203. Carbazole alkaloid murryaquinone A and related synthetic carbazolequinones as cytotoxic agents. J. Nat. Prod. 63(7):893-897.

Koster AS (1991). Bioreductive activation of quinones: a mixed blessing. Pharm. Weekbl. Sci. 13(3):123-126.

Leyva A, Pessoa C, Boogaerdt F, Sokaroski R, Lemos TL, Wetmore LA, Huruta RR, Moraes MO (2000). Oncocalyxones A and C, 1, 4-anthracenediones from *Auxemma oncocalyx*: comparison with anticancer 1,9-anthracenediones. Anticancer Res. 20(2A):1029-1031.

Liew FY (1992). Induction, regulation and function of T-cell subsets in leishmaniasis. Chem. Immunol. 54:117-135.

Lima HC, Bleyenberg JA, Titus RG (1997). A simple method for quantifying *Leishmania* in tissues of infected animals. Parasitol. Today 13(2):80-82.

Lima NM, Correia CS, Leon LL, Machado GM, Madeira Mde F, Santana AE, Goulart MO (2004). Antileishmanial activity of lapachol analogues. Mem. Inst. Oswaldo Cruz 99(7):757-761.

Machado PR, Ampuero J, Guimarães LH, Villasboas L, Rocha AT, Schriefer A, Sousa RS, Talhari A, Penna G, Carvalho EM (2010). Miltefosine in the treatment of cutaneous leishmaniasis caused by *Leishmania braziliensis* in Brazil: a randomized and controlled trial. PLoS Negl. Trop. Dis. 4(12):e912.

McSorley S, Proudfoot L, O'Donnell CA, Liew FY (1996). Immunology of murine leishmaniasis. Clin. Dermatol. 14(5):451-464.

Morello A, Pavani M, Garbarino JA, Chamy MC, Frey C, Mancilla J, Guerrero A, Repetto Y, Ferreira J (1995). Effects and mode of action of 1,4-naphthoquinones isolated from *Calceolaria sessilis* on tumoral cells and trypanosoma parasites. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 112(2):119-128.

Murray HW (1981). Susceptibility of *Leishmania* to oxygen intermediates and killing by normal macrophages. J. Exp. Med. 153(5):1302-1315.

Murray HW, Nathan CF (1999). Macrophage microbicidal mechanisms *in vivo*: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral *Leishmania donovani*. J. Exp. Med. 189(4):741-746.

Nestor KE Jr, Emmerson DA, Anthony NB, Nestor KE (1991). Research note: lack of an effect of high levels of menadione on tumor development in Japanese quail females. Poult. Sci. 70(11):2382-2385.

Oliveira CI, Nascimento IP, Barral A, Soto M, Barral-Netto M (2009). Challenges and perspectives in vaccination against leishmaniasis. Parasitol. Int. 58(4):319-324.

Pahn EM, Molina Portela MP, Stoppani AOM (1988). Effecto de quinonas y nitrofuranos sobre *Trypanosomas mega* y *Crithidia fasciculata*. Rev. Argent. Microbiol. 20(3):107-18.

Palumbo E (2009). Current treatment of cutaneous leishmaniasis: a review. Am. J. Ther. 16(2):178-182.

Panaro MA, Acquafredda A, Lisi S, Lofrumento DD, Trotta T, Satalino R, Saccia M, Mitolo V, Brandonisio O (1999). Inducible nitric oxide synthase and nitric oxide production in *Leishmania infantum*-infected human macrophages stimulated with interferon-gamma and bacterial lipopolysaccharide. Int. J. Clin. Lab. Res. 29(3):122-127.

Pearson RD, Queiroz Sousa A (2000). Leishmania species: visceral (kala-azar), cutaneous, and mucosal leishmaniasis. In: Mandell GL, Bennet EJ, Douglas R (eds). Principles and Practice of Infectious Diseases 5th ed. Churchill Livingstone, New York, USA. pp. 2832-2845.

Perry NB, Blunt JW, Munro MH (1991). A cytotoxic and antifungal 1,4-naphthoquinone and related compounds from a New Zealand brown alga, *Landsburghia quercifolia*. J. Nat. Prod. 54(4):978-85.

Pessoa ODL, De Lemos TLG, Silveira ER, Braz-Filho R (1993). Novel cordiachromes isolated from *Auxemma oncocalyx*. Nat. Prod. Lett. 2:145-150.

Pessoa ODL, De Lemos, TLG (1997). Allantoin and fatty acid composition in *Auxemma oncocalyx*. Rev. Bras. Farm. 78:9-10.

Piscopo TV, Mallia AC (2006). Leishmaniasis. Postgrad. Med. J. 82(972):649-57.

Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM (2005). A review of natural products with antileishmanial activity. Phytomedicine 12(6-7):514-535.

Sauvain M, Dedet JP, Kunesch N, Poisson J, Gantier JC, Gayral P, Kunesch G (1993). *In vitro* and *in vivo* leishmanicidal activities of natural and synthetic quinoids. Phytother. Res. 7(2):167-171.

Sittie AA, Lemmich E, Olsen CE, Hviid L, Kharazmi A, Nkrumah FK, Christensen SB (1999). Structure-activity studies: in vitro antileishmanial and malarial activity of anthraquinones from *Morinda lucida*. Planta Med. 65(3):259-61.

Sousa AQ, Frutuoso MS, Moraes EA, Pearson RD, Pompeu MM (2011). High-dose oral fluconazole therapy effective for cutaneous leishmaniasis due to *Leishmania (Vianna) braziliensis*. Clin. Infect. Dis. 53(7):693-5.

Taswell C (1984). Limiting dilution assay for the determination of immunocompetent cell frequencies III. Validity tests for the single-hit Poisson model. J. Immunol. Methods 72(1):29-40.

Teixeira MJ, de Almeida YM, Viana JR, Holanda Filha JG, Rodrigues TP, Prata JR Jr, Coêlho IC, Rao VS, Pompeu MM (2001). *In vitro* and *in vivo* leishmanicidal activity of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (lapachol). Phytother. Res. 15(1):44-48.

Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Muñoz V, Callapa J, Lobstein A, Anton R (2001). Antiprotozoal activities of Colombian plants. J. Ethnopharmacol. 78(2-3):193-200.

Wilson ME, Andersen KA, Britigan BE (1994). Response of *Leishmania chagasi* promastigotes to oxidant stress. Infect. Immunol. 62(11):5133-5141.

World Health Organization (WHO) (2010). Control of the Leishmaniasis, Technical Report Series No. 949. World Health Organization, Geneva. P 201.

Zarley JH, Britigan BE, Wilson ME (1991). Hydrogen peroxide mediated toxicity for *Leishmania donovani chagasi* promastigotes: role of hydroxyl radical and protection by heat shock. J. Clin. Investig. 88(5):1511-1521.